Our Reference No. 2223-171

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Confirmation No.: 5362

Appl. No.	:	10/731,741
Applicants	:	Schmitt et al.
Filed	:	December 10, 2003
Title	:	Cell Preparations Comprising Cells of the T Cell Lineage and Methods of Making and Using Same.
TC./A.U.	:	1632
Examiner	:	Anoop Kumar Singh
Docket No.	:	2223-171
Customer No.	:	001059

DECLARATION UNDER 37 C.F.R. 1.132

Honourable Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

Dear Sir:

I ,Ellen Rothenberg citizen of the United States of America and resident of California declare that the following facts are within my knowledge and are true.

1. I am a Professor of Biology at the California Institute of Technology. My research studies the molecular mechanisms that are responsible for developmental lineage choice as hematopoietic stem cells differentiate into T

lymphocytes. My laboratory focusses on identifying the transcription factors and signaling events that induce T-lineage gene expression in an uncommitted precursor and determining how they work to force the cell to relinquish other developmental options. My curriculum vitae is attached as Exhibit A.

2. I have reviewed the above-referenced application (hereinafter "the application") and the Office Action for the application that issued on October 27, 2008.

3. In particular, I note the Examiner's objection the claims as being obvious over a combination of the references Jaleco et al. (2001, J. Exp. Med. 194:991-1001, IDS); Nakano et al. (1994, Science 265:5157 IDS); Pui et al. (Immunity, 1999, 11(3):299-308), and Tatsumi et al. (1990, Proc. Natl. Acad. Sci. 87:2750-2754, IDS). I respectfully disagree with the Examiner for the reasons that follow.

4. For researchers working at the frontier of T-cell developmental biology in 2002, the publication by the inventors of the OP9-DL coculture method for T-cell generation in vitro was nothing short of a revolution. From the vantage point of hindsight in 2009, this impact may not be as clear as it was to everyone in the field at the time. The results of the inventors accomplished two completely novel things. One is that it decisively refuted the prevailing theory in the field, that T-cell development could only occur in a complex three-dimensional tissue (van

Ewijk et al., 1999- full citations in Appendix B). The other is that it provided for the first time a way to drive cells efficiently through successive stages of T-cell development through the full acquisition of cell-surface T-cell receptors under defined conditions. The effect of the work of the inventors was to transform the entire T-cell development field and to convert the T-cell development process from a difficult, irreproducible, laborious, and intractable problem to study into one of the most transparent and accessible of all mammalian developmental systems. The discontinuity was enormous.

5. The Examiner has adduced several papers that he believes weaken the case for the inventiveness of the OP9-DL coculture system. In particular, he points to the following, which were already known at the time: (1) the importance of Notch signaling for T-cell development and for suppression of B-cell development (Pui et al., 1999)[in fact, (Radtke et al., 1999) was also important to provide physiological relevance]; (2) the usefulness of the OP9 stromal cell line for eliciting B-cell development from embryonic stem cells (Nakano et al., 1994); and (3) the fact that some cells with cytoplasmic CD3 (a T- and NK-lineage marker) could be grown out of human precursor cells at the expense of B-cell development when the cells were cocultured with a different stromal line expressing Delta family but not Jagged family Notch ligands (Jaleco et al., 2001). He points to prior knowledge that different stromal lines could support different outcomes and contend that it was obvious to use Delta family molecules

in OP9 cells to solve the problem of in vitro T-cell development in 2002. In fact, while these foundational studies are relevant in a scholarly way to the generation of the OP9-DL system, they did not anticipate its effects.

6. Critically affecting the debate at the time were other features of the systems, not remarked by the Examiner. Knowledge of these other features actively held back other researchers from anticipating the success that the inventors in fact went on to obtain.

First, Notch signaling was already known to be complex, and not simply an instructive signal to make T cells. Abnormal Notch activation could cause T-cell development in abnormal sites (Pui et al., 1999), the biochemical events in this case were known not to be equivalent to Notch activation by interaction with normal Notch ligands. For example, although abnormally activated forms of Notch were able to affect certain aspects of T-cell development (the CD4/CD8 lineage choice), it was already known that normal Notch1 was not required for this event (Wolfer et al., 2001; Deftos et al., 2000; Robey et al., 1996), thus casting doubt on the biological relevance of artificial Notch activation such as that used by Pui et al. Second, Notch was known to affect many hematopoietic cell developmental decisions (Lam et al., 2000; Shelly et al., 2000; Tan-Pertel et al., 2000; Ohishi et al., 2000; Milner and Bigas, 1999; Li et al., 1998), not simply T-cell lineage choice. A particularly exciting recent finding in 2002 was the

ability of Notch pathway activation to sustain multipotent hematopoietic stem-cell-like activity (Varnum-Finney et al., 2000). Thus, even if Notch signaling enhanced T-cell development, it could do this in a very indirect way, simply by promoting the continued production of stem cells that had T-cell potential among other options. In this case, T cells might be produced only under conditions of Notch signaling, but they would be produced rather nonspecifically, together with many other cell types.

Second, T-cell development is a multi-step process that required an ordered progression of regulatory changes, not a simple "on" signal. This was evident from much research in the literature at the time, as I reviewed (Rothenberg, 2000), and was well supported by the stage-to-stage discontinuities in the roles of supporting cytokines (Di Santo et al., 1999) and intrinsic transcription factors (Engel et al., 2001). Any of a number of different regulatory deficiencies were known to block cells at early stages of this process and prevent generation of T-cell receptor-expressing cells (Staal et al., 2001). The expression of cytoplasmic CD3, such as that which is reported in Jaleco et al. (Jaleco et al., 2001), occurs at a relatively early stage of this process. It is not an indicator of success in generating immunologically significant, T-cell receptor-positive cells.

Third, T-cell development was known to fail on conventional stromal monolayer cocultures, even using thymic epithelial cells. This was a subject of much discussion at T-cell development meetings in the late 1990's and early 2000's. It was known that early T-cell development could proceed well in the three-dimensional structure of an organ culture but could not be sustained when the organ's own stromal cells were dissociated (Anderson et al., 1996; van Ewijk et al., 1999; Anderson and Jenkinson, 2001). This was not interpreted in terms of Notch ligand presentation, but rather in terms of the need for organized cell-cell interactions. The clear awareness that T-cell development depended on distinct, sequential regulatory signals as described above gave a simple explanation of why a complex architectural organization might be needed for correct guidance of development. This is relevant to answer the question of why it was not "obvious" to turn to OP9 cells as the solution to the T-cell problem. If even thymic epithelial cells themselves supported only partial, abortive T-cell differentiation, then why should any stromal line be better? If the problem was sequentiality of signals, then why should any homogeneous monolayer system work to support T-cell development for more than one or two stage transitions?

7. To summarize, the success of any attempt like that the inventors was viewed at the time as highly unlikely on the basis of these three facts about the complex roles of Notch, the discontinuous, multistep nature of T-cell

development, and the paradigm that 3-D organ structure was needed to induce and sustain T-cell development.

Each of the references cited by the Examiner added some background for the eventual success of the inventors, but they did not make it "obvious".

- The report by Jaleco et al. (Jaleco et al., 2001) was extremely important to show that different Notch ligands had different effects on the T and B lineage development of precursors, and to focus attention on Delta family ligands. However, the T-cell development that group obtained was extremely inefficient, rare, slow, and even questionable as to whether it was T-lineage at all. Knowing that the main problem in the T-cell field at the time was to generate a full multistage progression of T-lineage development, the tiny and slowly accumulating yield of potential T- and NK-lineage cells generated by Jaleco et al. did not make a serious impact.

- Similarly, it was not at all obvious why the OP9 stroma would do better than the S17 cells used by Jaleco et al. for this particular purpose. The feature of OP9 cells that was known was its lack of macrophage growth factor production; there was no evidence yet for any positive features they might have that would optimize T-cell development. The lack of macrophage growth factor was known to be helpful for B cell development from ES cells (Nakano et al., 1994), but it was not required to make B

cells from hematopoietic stem cells; indeed, the S17 cells used by Jaleco et al. were the standard for support of B cell development in vitro. Furthermore, while there was some precedent for hematopoietic precursors making binary choices between B and macrophage fates (Cumano et al., 1992), there was no conceptual foundation for why this should be an issue for T cells. In retrospect, OP9 cells are known to produce Wnt and Kit ligands that are likely to be critical for their support of T-cell development, but this was learned only after the fact.

An in vitro culture system in which a tiny percentage of cells adopt a partial, desired fate among a majority doing other things is not in any way the equivalent of an in vitro system that channels cells with high efficiency, reproducibility, and sustained effect throughout an extended differentiation process. The discovery that OP9-DL1 (or OP9-DL4) cells could indeed induce and sustain such a complex developmental program was not only a truly novel invention but also a paradigm-shattering discovery about the nature of T-cell development. The explosion of publications and the complete reorientation of the field that the inventors made possible are the highest expert testimony to its originality.

8. I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be

true and, further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such a willful false statement may jeopardize the validity of the application or any patent issuing thereon.

12 January 2009

Date

Ellen Rothenberg

Exhibit A

CURRICULUM VITAE

Ellen V. Rothenberg

Education:

Harvard University, Cambridge, Massachusetts
September 1969-June 1972
A.B. summa cum laude in Biochemical Sciences, 1972
Harvard University--Massachusetts Institute of Technology, Boston, Massachusetts
September 1972-January 1973
Joint program in Health Sciences and Technology
Courses toward M.D.
Massachusetts Institute of Technology, Cambridge, Massachusetts
Department of Biology and Center for Cancer Research
September 1972-January 1977
Ph.D. in Cell Biology, 1977 (advisor: David Baltimore)
Memorial Sloan-Kettering Cancer Center, New York, New York
Department of Cell Surface Immunogenetics
November 1977-September 1979
Postdoctoral (advisor: Edward A. Boyse)

Positions Held:

Teaching Assistant--Massachusetts Institute of Technology, General Biology (supervisor: Salvador Luria) February 1974-June 1974
NSF Predoctoral Research Fellow—MIT, September 1973-August 1976
Research Associate--Center for Cancer Research, Massachusetts Institute of Technology (advisor: David Baltimore), February 1977-October 1977
Postdoctoral Research Fellow--Memorial Sloan-Kettering Cancer Center, New York, New York, November 1977-September 1979
Assistant Research Professor--The Salk Institute for Biological Studies, Department of Cancer Biology, September 1979-May 1982
Assistant Professor of Biology--California Institute of Technology, Division of Biology, Pasadena, California, June 1982-June 1988
Associate Professor of Biology--California Institute of Technology, Division of Biology, Pasadena, California, July 1988-August 1994.
Professor of Biology--California Institute of Technology, Division of Biology, Pasadena, CA, September 1994-present
Albert Billings Ruddock Professor of Biology—California Institute of Technology, Division of Biology, Pasadena, CA, April 2007-present

Honors and Awards:

National Merit Scholar, 1969
Phi Beta Kappa, 1971
A.B. summa cum laude, Harvard University, 1972
National Science Foundation Predoctoral Fellowship, 1973-1976
Jane Coffin Childs Memorial Fund for Medical Research Postdoctoral Fellowship, 1977-1979
Biology Undergraduate Students Advisory Council Award for excellence in teaching, 1988
Ferguson Prize for Undergraduate Teaching, 1995
Associated Students of Caltech (ASCIT) Award for Excellence in Undergraduate Teaching, 1998
Ferguson Prize for Undergraduate Teaching, 1999
Biology Undergraduate Students Advisory Committee Award for Excellence in Teaching, 2001
Biology Undergraduate Students Advisory Committee Award for Excellence in Teaching, 2003-2004
Associated Students of Caltech (ASCIT) Award for Excellence in Undergraduate Teaching, 2007

Professional Societies:

American Society for Microbiology (former)
Sigma Xi (former)
AAAS (active)
American Association of Immunologists (active)
New York Academy of Sciences (former)

Committee Memberships and Editorial Boards:

Council Member--Midwinter Conference of Immunologists, 1/1981-1/1986
Member of review committee--American Cancer Society (California Division) Postdoctoral Fellowship Program, 9/1982-8/1985
Associate Editor, Journal of Molecular and Cellular Immunology, 1984-
Associate Editor, Journal of Immunology, 7/1986-6/1991
Associate Editor, Molecular Reproduction and Development, 1987-1997
Member, Awards Committee, American Association of Immunologists, 7/1986-6/1989
Member, Immunological Sciences Study Section, Division of Research Grants, National Institutes of Health/Public Health Service, 9/1988-6/1992
Program (Block) Chairman, American Association of Immunologists, T Cells (Block B), 1989-1992
Member of review committee--American Cancer Society (California Division) Postdoctoral Fellowship Screening Panel, 9/1992-7/1995 (2nd term)
American Institute of Biological Sciences Scientific Working Group and Peer Review Panel to NASA: Physiological and Anatomical Rodent Experiment.04, 1992-1994
Member, Scientific Advisory Board, Hereditary Disease Foundation, 5/1991-2/1995
Member, NIAID-NIA Task Force on Immunology and Aging, 1994
International Union of Immunology Societies designated reporter for 9th International Congress of Immunology, 1995
Member, National Research Council panel for review of Howard Hughes Medical Institute Predoctoral Fellowships in the Biological Sciences, 1996

Faculty, American Association of Immunologists Advanced Course in Immunology, July 1996
Editorial Board Member, Journal of Clinical Immunology, 1997-2000
Editorial Board Member, Developmental and Comparative Immunology, 1997-2000
Member, External Advisory Board, The Lerner Research Institute (Cancer Center), Cleveland Clinic Foundation, 1997-2003
Founding organizer, Aegean Conference on Gene Regulation in Lymphocyte Development, and co-chairman, 2002-2004
Faculty, Japanese Society for Immunology summer immunology course, 2004
Section Editor, *Journal of Immunology*, 2004-2006
Associate Editor, *Immunity*, January 2005 –
Scientific Advisory Board, La Jolla Institute for Allergy and Immunology: 2005 – present.
Faculty, AAI Advanced Immunology Course, July 2005, July 2006.
Program (Block) Co-Chairman, American Association of Immunologists, Hematopoiesis and Immune System Development, 2007-2010.
Editorial Board member, *Immunological Reviews*, 2009-2014.
Co-organizer, FASEB summer conference "Molecular Mechanisms of Lymphocyte Differentiation", 2009
14th International Congress of Immunology Program Committee, 2010

Site visitor and ad hoc reviewer for NCI, NIA, and CSR (NIH); NASA; other agencies

Publications:

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2. Haseltine, W. A., D. G. Kleid, A. Panet, E. Rothenberg and D. Baltimore. 1976. Ordered transcription of RNA tumor virus genomes. *J. Mol. Biol.* **106**, 109-131.

3. Rothenberg, E. and D. Baltimore. 1977. Increased length of DNA made by virions of murine leukemia virus at limiting magnesium ion concentration. *J. Virol.* **21**, 168-178.

4. Rothenberg, E., D. Smotkin, D. Baltimore and R. A. Weinberg. 1977. *In vitro* synthesis of infectious DNA of murine leukemia virus. *Nature* (London) **269**, 122-126.

5. Rothenberg, E., D. J. Donoghue and D. Baltimore. 1978. Analysis of a 5' leader sequence on murine leukemia virus 21S RNA; heteroduplex mapping with long reverse transcriptase products. *Cell* **13**, 435-451.

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12. Rothenberg, E. and D. Triglia. 1980. *In vitro* maintenance of differentiation marker synthesis by subpopulations of mouse thymocytes. *Proc. 1980 ICN-UCLA Symp. on Control of Cellular Division and Development and J. Supramolec. Struct.* **14**, 371-382.

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